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The purpose of this Center of Excellence is to bring molecular risk prediction for breast cancer into the clinical arena. Our specific aims are to establish a tissue repository from a retrospective cohort of women with benign breast disease (BBD) to identify those who developed breast cancer (cases) and those who did not (controls); to test potential biomarkers of risk in this archival tissue set; and to discover new, potentially relevant biomarkers of risk in fresh and frozen specimens of BBD.

We are completing follow-up on a retrospective cohort of 12,000 women with BBD diagnosed at Mayo Clinic between 1967 and 1991. We anticipate identifying approximately 700 cases and we will match appropriate controls to those cases. We have demonstrated excellent participation rates and access to benign tissues from cases and controls. We have information on established risk factors, permitting independent evaluation of the molecular markers. Upon finalization of all Human Subjects issues (approval still pending at Wayne State), we will initiate collection of fresh BBD samples for the discovery phase of our work.

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INTRODUCTION

The purpose of the Center of Excellence is to bring molecular risk prediction for breast cancer into the clinical arena. There are three main areas of scientific activity within this proposed Center: 1) the establishment of a large tissue repository from a retrospective cohort of women with BBD (1967-91) with complete and long-term clinical follow-up to identify those who developed breast cancer (cases) and those who did not (controls); 2) the application of potential biomarkers of risk to this archival tissue set; and, 3) the discovery of new, potentially relevant biomarkers of risk in fresh and frozen specimens of BBD. The Center includes a multi-institutional team of basic scientists, pathologists, epidemiologists, clinicians, statisticians, and advocates (Mayo Clinic; UCSF; Wayne State.

BODY

Task 1: Establish Retrospective Cohort of BBD and Nested Case-Control Study

Complete follow-up of 1982-91 group:

This work was initiated under a prior award from the Komen Foundation. When we submitted our Center grant, there were approximately 1500 individuals remaining to be followed. This work has been completed. Our approach and results are as follows:

Approach: Female patients who had a breast biopsy performed between January 1, 1982 and December 31, 1991 at the Mayo Clinic in Rochester, Minnesota (MCR) were identified using the resources of the Division of Medical Information Resources (MIR). For every surgical procedure performed at the MCR, MIR personnel review the surgical and pathology notes for the type of surgical procedure and diagnosis. The diagnostic data are coded using ICD-9 criteria and entered into a computer database referred to as the Surgical Index. A search of the Surgical Index identified 6,212 woman who had at least one breast biopsy during this time period.

The Pathology Index, a computerized database maintained by the Mayo Clinic Department of Laboratory Medicine and Pathology since 1985 containing all histologic findings for every surgery performed at the Mayo Clinic, was searched to determine the histologic findings associated with each breast biopsy. The histologic findings of all breast biopsies performed prior to 1985 were abstracted by a trained registered nurse from pre-1985 Pathology Index maintained on index cards by month and year of procedure. For each woman, the histology from their first breast biopsy was reviewed. If the biopsy indicated a malignancy or was performed as part of a prophylactic mastectomy, the patient was considered ineligible for the study. If the biopsy was performed for dermatologic reasons, the patient's next biopsy results were reviewed for eligibility. If no other biopsy was performed during this time period, the patient was considered ineligible. The first breast biopsy a woman had during this time frame was included in the next step in the screening process.

The next step in the screening process used two additional databases, the Medical Index and the Tumor Registry. MIR personnel maintain the Medical Index and Tumor Registry. Since 1975, the Medical Index has electronically captured the diagnostic findings of each patient's inpatient and outpatient episode of care. The Tumor Registry begun in 1971 contains the diagnostic findings, staging and follow-up status of patients diagnosed with cancer at our institution. Each of these databases were accessed to determine if any of the women considered potentially eligible from the previous screening step had had a cancer prior to her breast biopsy, had had a cancer diagnosed less than 6 months from the time of her breast biopsy, or had a diagnosis of breast cancer at least 6 months after her breast biopsy. Women found to have had a prior breast cancer or a diagnosis of breast cancer less than 6 months after her breast biopsy were considered ineligible. Those women found to have developed breast cancer at least 6 months after her breast biopsy were considered potential cases. At the end of this screening step, 5,340 women were considered eligible for the study.

Results: Surveys were mailed to these 5,340 women or their next-of-kin if the woman was known to be dead; 4354 (82%) of the surveys were returned. Six percent (n=319) of the women were lost to follow-up, 7% refused participation, and 5% could not be reached on telephone follow-up to complete the survey. The survey captured factors associated with an increased risk of breast cancer as well as information concerning additional breast biopsies and the development of breast cancer. We identified a total of 256 cases in the 1982-91 cohort.

Update follow-up of 1967-81 group

This cohort of women was originally established through an R01 grant to Dr. David Ballard (epidemiology) at Mayo Clinic in the late 1980s. This cohort includes 6,943 women with tissue available on 6,805 (98.1%). These women were last followed for subsequent occurrence of breast cancer through 1985; only 138 refused (2.0%) and 339 (5.0%) were lost to follow-up. A total of 294 cases of breast cancer were identified through 1985. Benign breast disease (BBD) tissue is available on 265 (90%) of these known cases. According to patient registration records, 85% of phase I cohort members are still living.

In our Center grant, we proposed to update the follow-up of these women. To do so, we required the approval of the DOD Human Subjects Committee. This was received on February 3, 2003 (e-mail notification from Ms. Shirley Roach appended – Appendix I). We have prepared the follow-up questionnaire for this group and it is attached (Appendix II). Since February 3, we reviewed all of the aforementioned Mayo indexes and identified an additional 95 breast cancer cases diagnosed since 1985. Thus, cohort I now has 389 cases among the participants. Charts were reviewed to validate all breast cancer diagnoses. For women in both study cohorts (i.e., across the entire 25-year period) who were diagnosed with breast cancer somewhere other than Mayo Clinic (n=118, 18%), a contact was initiated to obtain permission to access their medical records associated with their breast cancer diagnosis and their breast cancer tissue. To

date, we have received tissue on 50% (n=59) of the 118 women diagnosed elsewhere. No tissue was available for 13% of these women and permission was denied by 17% (n=20) women or their next-of-kin.

Match breast cancer controls to cases

The following matching procedure was performed independently for the two study periods, 1967 to 1981 and 1982 to 1991. Any eligible woman that developed breast cancer at least 6 months after breast biopsy was considered a case. Eligible women who did not develop breast cancer were considered controls.

One case was matched to 10 controls by age at breast biopsy and year of biopsy using the greedy matching algorithm developed by Rosenbaum. Ten controls were identified for each case to allow for the inclusion of 2 matches with available tissue. The closest matches will be used. The algorithm begins by randomly sorting the cases and controls. The distance between the first case and each potential control is determined using a weighted sum of the absolute difference between their age at biopsy and the year of their biopsy. All pairings where the follow-up time of the potential control is less than the time from breast biopsy to breast cancer diagnosis of the case is eliminated from consideration. The control closest to the case (i.e. the one with the smallest distance) is chosen as the case's first control. The algorithm then moves on the second case in the listing and computes the distance between that case and all remaining potential controls. This process continues until all cases have their first control. The second control is then found and the process is repeated a total of 10 times to obtain 10 matches of each case. The result of this allocation scheme is that the case-control matches on the first pass are the closest (in terms of age at biopsy and year of biopsy). With each successive pass, the distance between the case and additional control grows.

Construct test set for preliminary evaluation of biomarkers

A subset of approximately 125 cases and their two closest controls was chosen from the entire study period, 1967 to 1991, to serve as a test set. This set will be used to ascertain a point and interval estimate of the prevalence of a candidate marker among the cases, as well as the risk of breast cancer among those with the candidate marker relative to those without the marker. The most promising candidate markers will be assessed further in the validation data set.

The first step in constructing the test set was to determine the proportion of cases that occurred in each calendar year (1967-1991). The number of cases to be included in the test set for a particular year was that percentage of 125. Once the number to be selected from a particular calendar year was determined, that number was randomly selected from among the cases in that calendar year.

Task 2: Biomarkers In Archived Tissues from Cases and Controls

Retrieve tissue slides of BBD specimens for cases and controls

To date we have obtained benign tissue blocks for 589 of the 645 (91%) identified cases and 33% of their matched controls (this process is ongoing). Archived tissue of paraffin blocks and slides for these patients was obtained from the Mayo Clinic Tissue Registry. For tracking purposes, the pathology numbers, assigned at biopsy to the blocks and corresponding slides, are entered on SAS screens in the data set for each patient. A tracking system in the database identifies the location of those blocks and slides at any point in time (see description of database in Task 4 below).

Characterize benign histopathology

Our study pathologists, Drs. Carol Reynolds and Dan Visscher, have characterized the benign histology for 4,050 specimens to date. We are on target to complete the histopathology readings by the end of year 2. The general categories and proportions are: non-proliferative disease without atypia (66%); proliferative disease without atypia (28%); and proliferative disease with atypia (3%).

Prepare slides for biomarker analyses

Slide preparation will occur in the Tissue Acquisition Core of the Mayo Clinic Cancer Center (MCCC). As blocks need to be sectioned for slides, an identifying code number will be etched on the slides. A software system, using bar codes and bar code readers, to track the location of these slides and blocks has been purchased and installed. A pilot of this system has been tried and is working. (This process is described below in the relational database section.)

Many of the older tissues are embedded in paraffin blocks of odd size and/or the paraffin is too brittle to cut. This has required us to re-embed these older specimens. To date we have re-embedded 706 blocks in new paraffin, and continue to do so as blocks are identified.

Perform immunohistochemistry (IHC); perform FISH; perform centrosome studies

To avoid wastage of irreplaceable specimens from cases, we began our preliminary biomarker analyses in a separate "technical set" of benign breast specimens from women who were excluded from our study cohort for a variety of reasons (usually previous breast cancer).

We are using the technical set to determine the optimum staining and processing protocols for immunohistochemistry (IHC), immunofluorescence (IF) for centrosome analysis, and fluorescence in situ hybridization (FISH). The necessity of adding a technical set arose when discussing the length of time some of these tissues have been in paraffin. We wanted to verify that various biomarker analyses were viable across the entire time period. Together, the blocks in the technical set are representative of the

entire BBD cohort with regard to date of surgery (between 1967 and 1991) and with regard to pathology of the original BBD lesion and any subsequent malignant lesion.

IHC. We have optimized COX-2 and cyclin D1 labeling using the technical set and have determined that labeling is consistent across the four decades during which the cohorts were accrued (Figure 1A). The COX-2 protocol uses a 1:200 dilution of rabbit polyclonal antibody against COX-2 (Cayman Chemical, catalogue #160126) after EDTA antigen retrieval. The cyclin D1 protocol uses a 1:100 dilution of a rabbit polyclonal antibody against the C-terminus of human cyclin D1 (Biocare Medical, catalogue #CP236B after EDTA retrieval (Figure 1B). We are also exploring two dual labeling combinations in order to maximize the use of the smaller pieces of tissues in the cohort. To date we have tested p53 combined with Her2/neu and Ki67 (MIB-1) combined with EGFR (see Figure 1C). The labeling protocols for these combinations are currently being optimized using tissues known to be positive and will be tested on the technical set in the near future.

Centrosome studies. Gamma tubulin immunofluorescence labeling has been optimized and tested on 15 tissues from the technical set (Figure 1D). This protocol uses a 1:500 dilution of a monoclonal antibody against gamma-tubulin (Sigma Chemical Company, catalogue #T6557) after a proteinase K digestion. Images were collected and analyzed using a Zeiss LSM 510 scanning laser confocal microscope with a Meta detector. This method will be used for the centrosome analysis project.

FISH. A FISH protocol has been optimized using centromeric probes for chromosomes 3, 7 and 17. We have selected some locus-specific probes and will begin testing them in the near future.

Summary. To date we have cut more than 500 sections from the technical set in order to optimize conditions for IHC, IF, and FISH. We have optimized COX-2 labeling and are in the process of optimizing two dual label combination stains.

Task 3: Discovery - *In Vitro* Culturing and Gene Profiling Studies

Identify appropriate frozen specimens

Task 3 of our Center grant is directed toward the discovery of new, potentially meaningful biomarkers of risk. This work will be based in prospectively ascertained samples of benign breast disease from Mayo Clinic (Caucasian women) and Wayne State (African American women).

For Mayo, we have both institutional and DOD IRB approval to begin to collect such samples. Because of delays in this approval process (final approval received 2/3/03), we have not yet approached patients about participation. However, we have prepared the consent form (Appendix III) and protocol (Appendix IV) for the process. Those documents are appended. We will initiate patient accrual this summer.

For Wayne State, there has been a delay in their obtaining IRB approval to proceed. This delay hinged on the DOD's previous requirement that patients or their insurors not be charged for any possible research-related risks. This discussion consumed untold hours of work on the part of numerous individuals. It now appears that a solution is in sight, but the documents are still under review at the Wayne State IRB.

Obtain fresh BBD tissue from patients

As was described in the previous section, we have not yet initiated patient accrual but will do so in the next month or two.

Culture 80 BBD specimens and document growth characteristics

See preceding sections. The culturing will follow, as soon as the fresh specimens are obtained.

Compare genomic expression levels of DCIS markers in BBD tissues

We have identified 10 cell cycle and cytoskeletal genes (TTK, Cen2, EB1, CD27, GST1, TACC3, SEI1, BCCIP, skb1 homologue, and PCR1) that are over-expressed in DCIS relative to normal breast to use in quantitative real-time PCR studies of benign tissues that will be collected prospectively. Laser capture microdissection will be used to collect benign epithelial cells from which RNA will be isolated. We also have collected 10 fresh frozen DCIS tissues from which we will isolate RNA for gene expression studies. In these studies, we will use Affymetrix gene chips to compare gene expression in DCIS relative to 5 normal breast tissues in order to identify additional genes for real-time PCR of benign breast lesions.

Task 4: Statistical Analyses

Establish relational database

We created a Sybase database to track tissue (paraffin embedded blocks, tissue slides and pieces of tissue) samples as they are moved between laboratory locations and to manage the biomarker result data. A web-based interface to this database has been created. All samples (individually or in boxes) are tracked using bar codes. The interface allows users to scan the bar code of the sample labels and enter information as they perform a task such as moving a sample, creating a slide, or entering laboratory results.

The process begins as the information on the paraffin blocks is stored in the database. These blocks are inserted into barcode labeled boxes which are scanned whenever they are moved to a new location. In a processing laboratory, the blocks are cut and the tissue slices are affixed to barcode labeled slides or – for very thick slices – placed into vials. The slides are scanned as they are put into slide boxes. The boxes in turn are scanned as they move to new locations and when identical laboratory procedures

(e.g., a tissue stain) are done to all samples in the boxes. Each activity is stored in the database along with the time of the activity and the identification number of the person who performed the activity.

At the time of sample analysis, the physician, researcher or laboratory technician scans the samples and uses the same web-based application to enter the results of the analysis. All results and transactional information are stored in the database and available for statistical analysis.

The database server software is the current Sybase relational database management system. The data model was created and managed with Sybase PowerDesigner. The users will access the database via the Mayo internal web using programs written in Cold Fusion. The data analysts will access the database using connections to the SAS statistics analysis system. ODBC connections are used to connect web forms and the SAS system to the database.

The components of the database are pictured in Figure 2. The central table in the database is the "sample" table which contains links to most of the other tables such as the "patient" table (containing patient information) , the "block" table (containing information about the paraffin block from which the sample was cut), the "box" table (showing the current location of the sample), the "stain" table (showing stains done to the sample), the "results" table (containing the results of many types of tests done to each sample) and the "fishrslt" table (a table for FISH results). Most of these secondary tables contain links to descriptive tables, such as the "location" table and the "test_type" table, which manage the drop-down selection boxes in the web pages.

It is estimated that the database will contain information for approximately 12,000 patients, 300,000 samples and the test results for each of the samples.

Data entry

For the 1982-91 cohort, data from all 4354 questionnaires have been entered into the database. Along with these questionnaire data, we have also updated contact information for the women or their next-of-kin.

Other data entry activities include:

- Entry of 1400 of the 4050 histopathology readings
- Entry of slides into the database as we receive them from tissue registry and prior to sending them to Dr. Visscher
- Entry of available tissue blocks into the database
- Documentation of breast cancers. We verified the breast cancer by medical records and recorded the histopathology, TNM, date of diagnosis, recurrence information when available, ER, PR, and type of surgery.

KEY RESEARCH ACCOMPLISHMENTS

We are in the first year of our award – our Human Subjects approval at Mayo was received just over four months ago (2/4/03) and such approval is still pending at Wayne State. At this time, we do not have research accomplishments per se to report.

REPORTABLE OUTCOMES

- Abstract, AACR, 2003 by Hartmann et al on the 1982-91 cohort (Appendix V)
- Construction of BBD tissue repository across entire 25 yr time period, 1967 - 1991
- Development of relational database

CONCLUSIONS

Optimal early detection and prevention strategies for breast cancer are predicated on our ability to identify individuals at significantly increased risk for this disease. Unfortunately only a minority of the ~ 200,000 women who are diagnosed with breast cancer in the US each year are recognized as being at significantly increased risk. The purpose of this Center is to bring molecular risk prediction for breast cancer into the clinical arena. This will require progress on three fronts of scientific endeavor: (i) Establishment of a tissue repository of benign breast disease; (ii) Assessment of potential biomarkers of risk in this tissue set and (iii) Discovery of new, potentially relevant biomarkers of risk. We have made good progress in these first months on our tissue repository and preparation for our biomarker studies. With the recent approval of our Mayo Human Subjects work (and the anticipated approval at Wayne State), we can begin the discovery work on prospectively ascertained samples.

REFERENCES

None.

APPENDICES

Appendix I

Hartmann, Lynn C., M.D.

From: Hartmann, Lynn C., M.D.
Sent: Wednesday, June 25, 2003 7:38 AM
To: Hartmann, Lynn C., M.D.
Subject: FW: SUBJECT: Protocol Entitled "Benign Breast Disease: Toward Molecular Prediction of Breast Cancer Risk", Submitted by Lynn C. Hartmann, M.D., Mayo Clinic Foundation, Proposal Log Number BC013015, Award Number DAMD17-02-1-0473, HSRRB Log Number A-11587.a

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From: Roach, Shirley A Ms AMDEX[SMTP:Shirley.Roach@DET.AMEDD.ARMY.MIL]
Sent: Monday, February 03, 2003 6:46 AM
To: 'Hartmann, Lynn C., M.D.'
Subject: SUBJECT: Protocol Entitled "Benign Breast Disease: Toward Molecular Prediction of Breast Cancer Risk", Submitted by Lynn C. Hartmann, M.D., Mayo Clinic Foundation, Proposal Log Number BC013015, Award Number DAMD17-02-1-0473, HSRRB Log Number A-11587.a

SUBJECT: Protocol Entitled "Benign Breast Disease: Toward Molecular Prediction of Breast Cancer Risk", Submitted by Lynn C. Hartmann, M.D., Mayo Clinic Foundation, Proposal Log Number BC013015, Award Number DAMD17-02-1-0473, HSRRB Log Number A-11587.a

Dear Dr. Hartman,

Approval for your site for the subject study was issued to the contracting office. Feel free to call me if you have any questions. The additional site will have problems getting approved unless there is modification of the medical care clause.

Sincerely,
Shirley A. Roach, ADN, BA, CCRP
Human Subjects Protection Scientist
AMDEX Corporation
Office of Regulatory Compliance and Quality
US Army Medical Research and Materiel Command
Phone: (301)-619-6238
DSN: 343-6238
Fax: (301) 619-7803

Official Address:
Commanding General
US Army Medical Research and Materiel Command
Attn: MCMR-RCQ-HR (Ms. Roach)
504 Scott Street
Fort Detrick, MD 21702-5012

Appendix II

1. Have you ever been diagnosed with breast cancer?

○ No ○ Yes

If yes, how old were you when you were diagnosed with breast cancer? (If more than one breast cancer, age at first diagnosis.)

Age

Which breast?

○ Left

○ Right

○ Both

2. **Have you had either breast removed?**

○ No ○ Yes

Please indicate which breast(s) were removed.

○ Left ○ Right ○ Both

At what age were your breast(s) removed?

Left breast Age

Right breast Age

What was the reason for having your breast(s) removed? (Mark all that apply.)

Left breast	Right breast
○ Breast cancer	○ Breast cancer
○ Risk of breast cancer	○ Risk of breast cancer
○ Treatment for breast problems	○ Treatment for breast problems
○ Other, specify: _____	○ Other, specify: _____

3. **Have your ovaries been surgically removed?**

○ No ○ Yes, one ovary ○ Yes, both ovaries ○ Yes, but unsure if one or both

At what age was your ovary/ovaries removed? (If more than one surgery, please record age at last surgery.)

Age

4. **Have you ever taken tamoxifen or raloxifene (Evista)?** (Mark all that apply.)

○ No ○ Yes, tamoxifen ○ Yes, raloxifene (Evista)

If yes, what year did you start taking tamoxifen?

Year

How many total years did you take tamoxifen?

○ Less than 1 year
○ 1 to 2 years
○ 3 to 4 years
○ 5 years
○ Over 5 years

If yes, what year did you start taking raloxifene (Evista)?

Year

How many total years did you take raloxifene (Evista)?

○ Less than 1 year
○ 1 to 2 years
○ 3 to 4 years
○ 5 years
○ Over 5 years

~~15~~ 16. Have you ever been diagnosed with cancer anywhere other than in the breast?

○ No ○ Yes

If yes, what type of cancer have you had?

○ Colon ○ Ovarian ○ Uterine ○ Other, specify: _____

At what age were you diagnosed?

Age | Age | Age | Age

~~17~~ 16. Have any of your blood relatives had breast cancer, prostate cancer, colon cancer, or ovarian cancer?

○ No

○ Yes

If yes, be sure to mark the space for all blood relatives and each specific cancer. For sisters, daughters, and aunts, please indicate the number who have breast cancer in each group (4+ means 4 or more of these people have breast cancer).

Cancer:	Your Sister(s)	Your Brother(s)	Your Daughter(s)	Your Son(s)
Colon	○	○	○	○
Ovarian	○		○	
Prostate		○		○
Breast	○1 ○2 ○3 ○4+	○	○1 ○2 ○3 ○4+	○
Age at diagnosis of breast cancer. (If more than one sister or daughter, age of youngest when diagnosed.)	Age		Age	

Continued next column...

Cancer:	Your Mother	Your Mother's Sister(s)	Your Mother's Brother(s)	Your Cousin(s) (Mother's Side)
Colon	○	○	○	○
Ovarian	○	○		○
Prostate			○	○
Breast	○	○1 ○2 ○3 ○4+	○	○1 ○2 ○3 ○4+
Age at diagnosis of breast cancer. (If more than one aunt or cousin, age of youngest when diagnosed.)	Age	Age		Age

Cancer:	Your Father	Your Father's Sister(s)	Your Father's Brother(s)	Your Cousin(s) (Father's Side)
Colon	○	○	○	○
Ovarian		○		○
Prostate	○		○	○
Breast	○	○1 ○2 ○3 ○4+	○	○1 ○2 ○3 ○4+
Age at diagnosis of breast cancer. (If more than one aunt or cousin, age of youngest when diagnosed.)		Age		Age

7. Have other cancers occurred in blood relatives on your mother's side?

○ No ○ Yes

If yes, please list relative and type of cancer.

_____

_____

_____

8. Have other cancers occurred in blood relatives on your father's side?

○ No ○ Yes

If yes, please list relative and type of cancer.

_____

_____

_____

If we need more information or have questions, please provide:

Name of Person Completing Survey:

_____
First Name

_____
Last Name

Phone numbers:

(___) ___ - ____
Area Code Number

(___) ___ - ____
Area Code Number

Best times to call

__:__ ○ a ○ p

__:__ ○ a ○ p

Appendix III

MAYO CLINIC
Rochester, Minnesota

Retain in Correspondence Section of Medical Record

Name and Registration No.

Consent Form for Participation in a Research Study

TITLE: "Study of Benign Breast Disease"

IRB#: 170-02 00

RESEARCHER: Dr. L. Hartmann and colleagues

PROTOCOL LAST APPROVED BY INSTITUTIONAL REVIEW BOARD: December 31, 2002

THIS FORM APPROVED: March 3, 2003

This is an important form. Please read it carefully. It tells you what you need to know about this research study. If you agree to take part in this research study, you need to sign this form. Your signature means that you have been told about the study and what the risks are. Your signature on this form also means that you want to take part in this study.

Why is this research study being done?

This study is being done to study what regulates breast tissue growth, to better understand if there is a possible relationship between benign changes and breast cancer. Laboratory studies will search for changes in genetic material (DNA and chromosomes) and changes in other biologic processes in tissue specimens.

How many people will take part in this research study?

The plan is to have approximately 160 women take part in this study. Approximately 80 people will take part at Mayo Clinic Rochester and 80 from Wayne State Medical Center in Detroit.

What will happen in this research study?

At the time of your breast surgery, excess tissue may be available for use for research purposes. For some women, all the tissue is needed for diagnostic purposes (if this is the case with you, then your specimen would not be included in this study). In other women, the resected specimen contains more material than the pathologist needs to make the diagnosis. In this case, we would like to use this excess, already resected, tissue for research purposes. This research involves doing a number of studies in the laboratory, including studying the growth characteristics of breast tissue. Some of these laboratory studies will be performed by Dr. Thea Tlsty, a breast researcher at the University of California, San Francisco. Specimens from both Mayo Clinic Rochester and Wayne State will be shipped to Dr. Tlsty's laboratory for study. In addition, we ask that you complete a one-time questionnaire designed for this study. This should take approximately 10 minutes of your time to complete.

How long will I be in this research study?

Your breast tissue and information that you have provided on the study questionnaire will be used throughout the period of this study, anticipated to last approximately four years. If you reply that you will allow your tissue to be used in future studies (see below), it will be stored in a secure area of the Mayo Clinic. It may be stored indefinitely. If you don't want your tissue to be used in future studies, it will be stored in a secure area for five years after the completion of the study at which time it will be disposed per institutional policy for disposal of tissue. The five-year period allows time for validation of research findings. Questionnaire data will be stored in locked file cabinets in the research area or archival area of the institution for a period of five years. Electronically stored data will be stored in a secured location. Storage of research data for a period of five years is common. This allows the ability to reevaluate and validate findings.

Are there reasons I might leave this research study early?

Taking part in this research study is your decision. You may decide to stop at any time. You should tell the researcher if you decide to stop and you will be advised whether any additional tests may need to be done for your safety.

In addition, the researchers or Mayo may stop you from taking part in this study at any time if it is in your best interest, if you do not follow the study rules, or if the study is stopped.

Will any biological sample(s) be stored and used for research in the future?

If there is excess material available, your sample will be kept at Mayo for possible future use. In addition, researchers at Mayo who are not involved with this study may ask to use your sample for future research. You have a say in how your stored sample would be used in future research. You can still take part in the current study without giving your sample for future use. There are exceptions to use of your sample without your permission. These exceptions are: 1) when government rules allow your sample to be used without identifying you, even with a code; and 2) when use of the sample is not considered human subject research. At all other times:

- you can let Mayo use your sample; or
- you can say no to having your sample used by Mayo.

If you agree to allow your sample to be used for further research, the sample may be stored indefinitely. The sample will be stored at Mayo and would be given a code (rather than your name) while it is stored and when it is used in research. This code would allow your sample to be used without anyone knowing that it is your sample just by looking at the label.

There is a very small chance that some commercial value may result from the use of your sample. If that would happen, you will not be offered a share in any profits.

Please read the following statements and mark your choice:

1. I permit my sample to be stored and used in future research of breast tissue at Mayo:

☐ Yes ☐ No Please initial here:_____ Date:_____

2. I permit my sample to be stored and used in future research at Mayo to learn about, prevent, or treat any other health problems:

☐ Yes ☐ No Please initial here:_____ Date:_____

If you want your sample destroyed at any time, write to the Administrator of the Mayo Foundation Office for Human Research Protection, 201 Building 4-60, 200 First Street Southwest, Rochester, Minnesota 55905. Mayo has the right to end storage of the sample without telling you. If you move please send your new address to Mayo Clinic Rochester, Section of Registration, 200 First Street Southwest, Rochester, Minnesota 55905.

If you agree to give your sample, it will be the property of Mayo and may be used for research by Dr. Hartmann and other staff at Mayo Clinic. Researchers at other institutions also may one day ask for a part of your sample for other studies.

How do researchers from other institutions get the sample?

Researchers from universities, hospitals, and other health organizations conduct research using tissue. They may contact Mayo and request samples for their studies. If you approve release of your sample by checking 'yes' below, Mayo may send the tissue sample(s) and some information about you to researchers who request them, but Mayo will not send your name, address, phone number, social security number, or any other identifying information with the sample. If you allow your sample to be given to researchers at other institutions, it will be given to them with a code number rather than your name. If these researchers use the sample for future research and decide that a test result may be useful for your health care, they may contact the Mayo Clinic and Mayo would then contact you to offer you the choice to learn the test results.

I permit Mayo to give my sample to researchers at other institutions:

Please mark one box:
☐ Yes ☐ No Please initial here:_____ Date:_____

What are the risks of this research study?

You are being asked to let researchers use breast tissue that has already been removed. There are no recognized risks to allowing the use of this material for research.

Are there benefits to taking part in this research study?

This study will not make your health better. The current laboratory studies that will be performed on your specimen(s) are completely investigational at this time. There will be no direct health benefits to you resulting from these studies. The results of this study may improve the medical community's understanding of breast disease such that women in the future may benefit from this work.

What other choices do I have if I don't take part in this research study?

This study is only being done to gather information. You may choose not to take part in this study.

Will I need to pay for the tests and procedures?

You will not need to pay for any tests and procedures which are done just for this research study. However, you and/or your health plan will need to pay for all other tests and procedures that you would normally have as part of your regular medical care. Other than medical care that may be provided and any other payment specifically stated in the consent form, there is no other compensation available for your participation in this research.

What happens if I am injured because I took part in this research study?

There is no risk of physical harm when taking part in this study.

What are my rights if I take part in this research study?

Taking part in this research study does not take away any other rights or benefits you might have if you did not take part in the study. Taking part in this study does not give you any special privileges. You will not be penalized in any way if you decide not to take part or if you stop after you start the study. Specifically, you do not have to be in this study to receive or continue to receive medical care from Mayo Clinic.

You will be told of important new findings or any changes in the study or procedures that may affect you or your willingness to continue in the study.

Who can answer my questions?

You may talk to Dr. Lynn Hartmann at any time about any questions or concerns you have on this study. You may contact Dr. Hartmann (or an associate) by calling the Mayo operator at telephone (507) 284-2511.

You can get more information about Mayo policies, the conduct of this study, or the rights of research participants from Cindy L. Boyer, Administrator of the Mayo Foundation Office for Human Research Protection, telephone (507) 284-2329 or toll free (866) 273-4681.

Authorization To Use And Disclose Protected Health Information

By signing this form, you authorize Mayo Clinic Rochester and the investigators to use and disclose any information created or collected in the course of your participation in this research protocol.

This information may include information relating to sexually transmitted disease, acquired immunodeficiency syndrome (AIDS), or human immunodeficiency virus (HIV). It may also include information relating to behavioral or mental health services or treatment and treatment for substance abuse.

This information may be given to other researchers in this study (including those at other institutions), representatives of the company sponsoring the study, or private, state or federal government parties responsible for overseeing this research. These may include the Food and Drug Administration, the Office for Human Research Protections or other offices within the Department of Health and Human Services, and the Mayo Foundation Office for Human Research Protections or other Mayo groups involved in protecting research subjects.

This information will be given out for the proper monitoring of the study, checking the accuracy of study data, analyzing the study data, and other purposes necessary for the proper conduct and reporting of this study.

This authorization lasts forever.

You may stop this authorization at any time except if Mayo Clinic Rochester needs information already collected to ensure complete and accurate study results. This might mean that Mayo may continue to use your information collected as part of this study even after you have told us to stop. If this is a research study that also involves treatment, you may no longer be eligible to receive study treatment if you tell Mayo to stop using this information. The only way you can tell Mayo to stop using the information is in writing addressed as follows:

Mayo Foundation
Office for Human Research Protections
ATTN: Notice of Revocation of Authorization
200 First St. SW
Rochester, MN 55905

If this information is given out to someone else, the information may no longer be protected by federal privacy regulations and may be given out by the person or entity that receives the information.

A copy of this form will be placed in your medical record.

I have had an opportunity to have my questions answered. I have been given a copy of this form. I agree to take part in this research study.

(Date)	(Printed Name of Participant)	(Clinic Number)

(Signature of Participant)

(Date)	(Printed Name of Individual Obtaining Consent)

(Signature of Individual Obtaining Consent)

Appendix IV

2. For the *in vitro* culturing experiments in Aim 3 we will study prospectively ascertained specimens of BBD (Mayo IRB #170-02).

Recruitment Process, Mayo Clinic: Potential participants for the prospective cohort will be identified within our Breast Clinic. This facility provides an entry point for women with a new breast concern, either palpable or mammographically detected. At the Breast Clinic individuals are seen by a multi-disciplinary team as needed to address their specific problem. The Mayo Clinic Cancer Center's Breast Program supports a study assistant who identifies appropriate patients for a variety of studies in the Breast Clinic. For the current study, the study assistant will identify women who are going to have an excisional biopsy for a breast concern in the pre-op setting. To be included in the study, the women need to be between the ages of 18 and 100 years, not have a prior diagnosis of breast cancer, and not scheduled for a concurrent prophylactic mastectomy. The study assistant will determine patient eligibility using the following procedure: (a) medical records will be reviewed on all women scheduled in the Breast Clinic/Breast Center to determine eligibility of the patient, and (b) discuss eligibility that is uncertain with the attending physician or advanced practice nurse. The study assistant will explain the intent of the study. If a woman consents to participate, she will be asked to complete the survey while she is still in the Breast Clinic. Her specimen will be tracked at the Frozen Section Lab. If the specimen is benign on frozen section, and there is excess material not required for the diagnosis, a sample will be shipped to UCSF for culturing.

Frozen Tissue Review: For the culturing experiments, we intend to grow a total of 40 samples from Caucasian women over the period of the grant. Another 40 specimens from African-American women will be grown; these will be obtained from Wayne State. Several procedures will be followed in order to assure that occult, microscopic malignant lesions are not "missed" (i.e. lost, thus resulting in a false negative pathologic diagnosis) during the process of harvesting fresh tissue for cell culture studies. First, all samples will be obtained in the Frozen Section Lab under direct supervision of a pathologist. Second, tissue samples will routinely be obtained from grossly benign tissue in portions of the biopsy located at least 2 cm away from any grossly or radiographically suspicious areas. Since gross - radiographic correlation is performed immediately, in consultation with surgeons, any diagnostic abnormalities will be detected prior to partitioning for special studies. Finally, a "mirror image" section, obtained immediately adjacent to the partitioned sample, will be studied microscopically prior to releasing the specimen for cell cultures.

Recruitment Process, Wayne State: Potential participants will be identified within the Walt Breast Center. The Alexander J. Walt Comprehensive Breast Center is part of the Karmanos Cancer Institute, affiliated with Wayne State University. Also, Karmanos Cancer Institute is part of the Detroit Medical

Center. The Walt Breast Center is the entry point at Wayne State for women with new breast concerns. The multidisciplinary team includes research and nursing staff representative of the indigenous urban population. Fifty percent of the patients seen at the Walt Breast Center are African American. For the current study, the study assistant will identify African American women who are going to have an excisional biopsy for a breast concern. To be included in the study, the women need to be between the ages of 18 and 100 years, not have a prior diagnosis of breast cancer, and not scheduled for a concurrent prophylactic mastectomy. The study assistant will determine patient eligibility using the following procedure: (a) medical records will be reviewed on all women scheduled in the Breast Clinic/Breast Center to determine eligibility of the patient, and (b) discuss eligibility that is uncertain with the attending physician or advanced practice nurse. The study assistant will explain the intent of the study (see Informed Consent process below). If a woman consents to participate, she will be asked to complete the survey while she is still in the Breast Center. Her specimen will be tracked at the Frozen Section Lab using the same procedures outlined for the Mayo Clinic samples (see Frozen Tissue Review above). The Wayne State study assistant will track this tissue to the Frozen Section Lab. If the specimen is benign on frozen section, and there is excess material not required for the diagnosis, a sample will be labeled with a code identified by Wayne State investigators. The label will include the source of the tissue (e.g. breast) and date of biopsy. The tissue will then be shipped to UCSF. The questionnaire information will at that time also be labeled with the patient's code number and mailed to the Mayo Clinic study coordinator. Wayne State will keep a master list that connects code numbers with patient identification. This list will be kept in a database that is secured to use only by the Wayne State investigator and her study assistant. Questionnaires will be stored in a locked file cabinet until sent to Mayo Clinic.

It should be noted that our main study pathologist, Dr. Visscher, was recruited to Mayo from Wayne State. He is very familiar with the systems and procedures of both institutions and maintains close working relationships with members of the Wayne State Surgical and Pathology Departments. Dr. Visscher will assure standardization of tissue collection, reading, and processing at both sites.

The gene profiling experiments will be performed in 30 previously stored, frozen samples of BBD (covered under Mayo IRB #189-94). The 30 frozen samples are being stored in a freezer in the Pathology Department at Mayo Clinic. An approach where direct and indirect identifiers are stripped from the sample source with no means of linking to the sample source will be used for these samples. The process that will be used, consistent with Mayo Clinic IRB policy, includes collecting all the needed demographic and clinical information from the record (this will include age, ethnicity, and family history (no names will be abstracted). Identifiers will then be stripped from

the BBD sample and abstracted data. It will only be after all identifiers are stripped that we will perform any analysis on the sample. See Appendix G for Mayo Foundation IRB policy. We will not be administering a questionnaire to this group.

Appendix V

Benign Breast Disease and Breast Cancer Risk. LC Hartmann[1], D Visscher[1], C Reynolds[1], MH Frost[1], LJ Melton[1], C Vachon[1], T Tlsty[2], D Hillman[1], JL Johnson[1], WL Lingle[1], V Suman[1], TA Sellers[1].

[1] Mayo Clinic Cancer Center, Rochester, MN
[2] University of California, San Francisco, CA

Introduction: Benign breast disease (BBD) is an established risk factor for breast cancer (BC), but only a minority of women with BBD ultimately develop BC. The ability to identify the subset of women at greatest risk for breast cancer at the time of BBD diagnosis would permit more aggressive clinical intervention, including closer surveillance and prevention opportunities. To facilitate this discovery process, we have established a large historical cohort of women with BBD in which we can test more specific means of risk prediction, using clinical, histopathologic and molecular tools.

Methods: The Mayo Clinic Surgical Index was used to identify all women who had an open breast biopsy with benign findings at the Mayo Clinic between 1/1/82 and 12/31/91 (n = 5153). The availability of tissue slides and blocks on these patients was verified through linkage to the Pathology Index. Medical record review was performed to verify eligibility and to identify subsequent occurrences of breast cancer diagnosed or treated at Mayo. A study-specific questionnaire was mailed to collect risk factor data on the cohort and to identify breast cancers diagnosed outside of Mayo.

Results: This 10-year cohort includes 5153 women with 66,290 person years of follow-up (through 2/02). The median age at BBD diagnosis was 54 years (13-94), and 41% were age 50 or less. Some family history of breast cancer was present in 32%, while 17% had an affected first-degree relative. Thus far, 255 women are known to have developed BC. The interval from BBD to BC is: $\leq$ 5 yrs, 33.7%; 5.1-10 yrs, 34.5 %; 10.1-15 yrs, 27.5%; > 15 yrs, 4.3%. The cancer occurred in the same breast as the BBD in 125 women (49%), the opposite breast in 84 (32.9%), and both breasts in 10 (3.9%). Side of BC is pending for 36 (14%) women. The estimated 5-year, 10-year and 15-year breast cancer incidence rates are 1.8% (95%CI: 1.4-2.1%), 3.6% (95%CI: 3.1-4.2%), and 5.8% (95%CI: 5.1-6.5%), respectively. Incorporating time from BBD to cancer and the side of BBD vs BC, we are exploring a panel of biomarkers as indicators of possible BC precursors or a background field change.

Conclusions: We have assembled a large cohort of patients with BBD with extensive follow-up for breast cancer, excellent participation on a risk factor survey, and sufficient quantities of well-characterized tissues to permit independent evaluation of established and novel molecular markers.

Supported by grants from the national Komen Foundation, the Breast Cancer Research Foundation, and DOD Breast Cancer Center of Excellence award DAMD 17-02-1-0473.

Figure 1

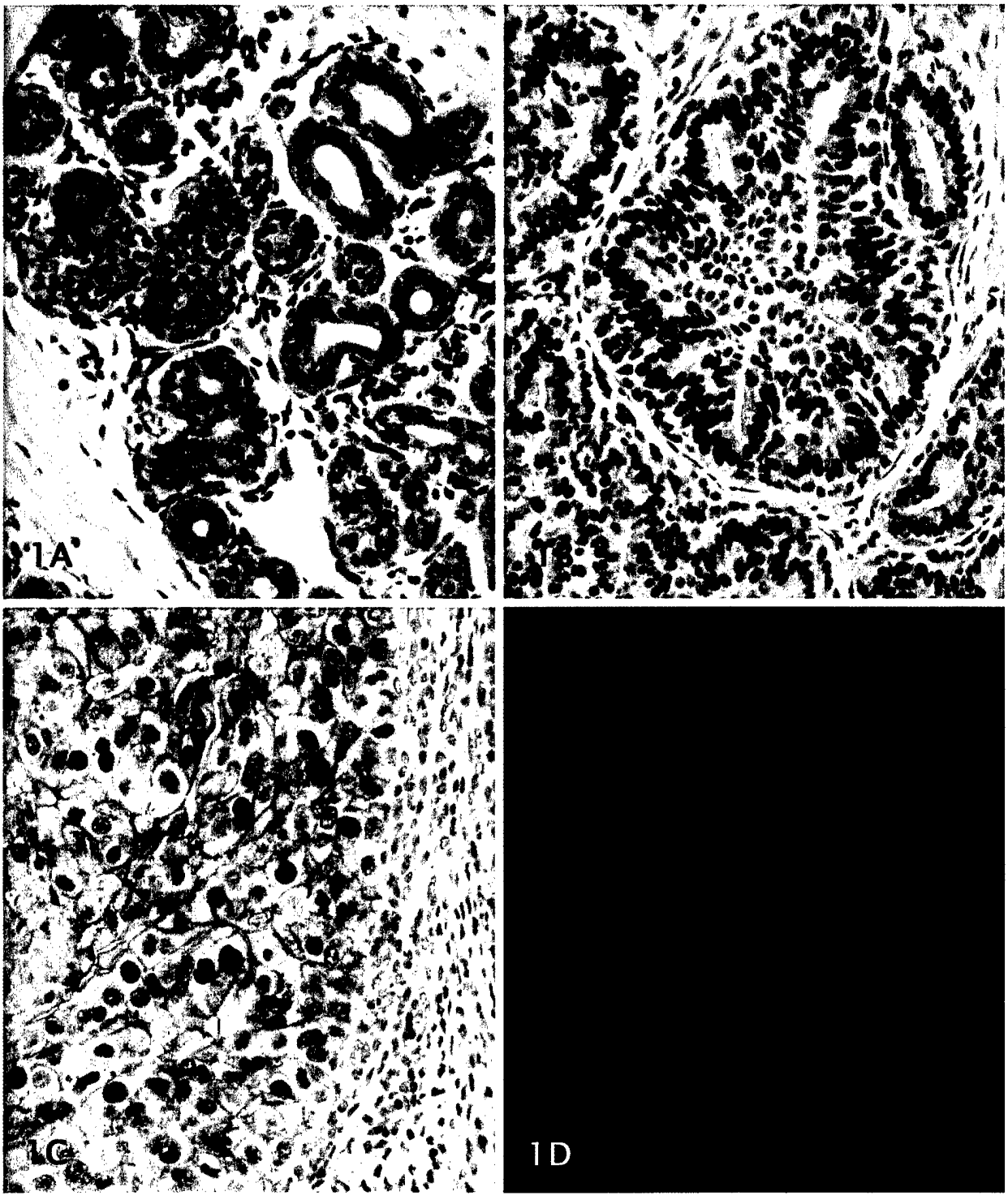
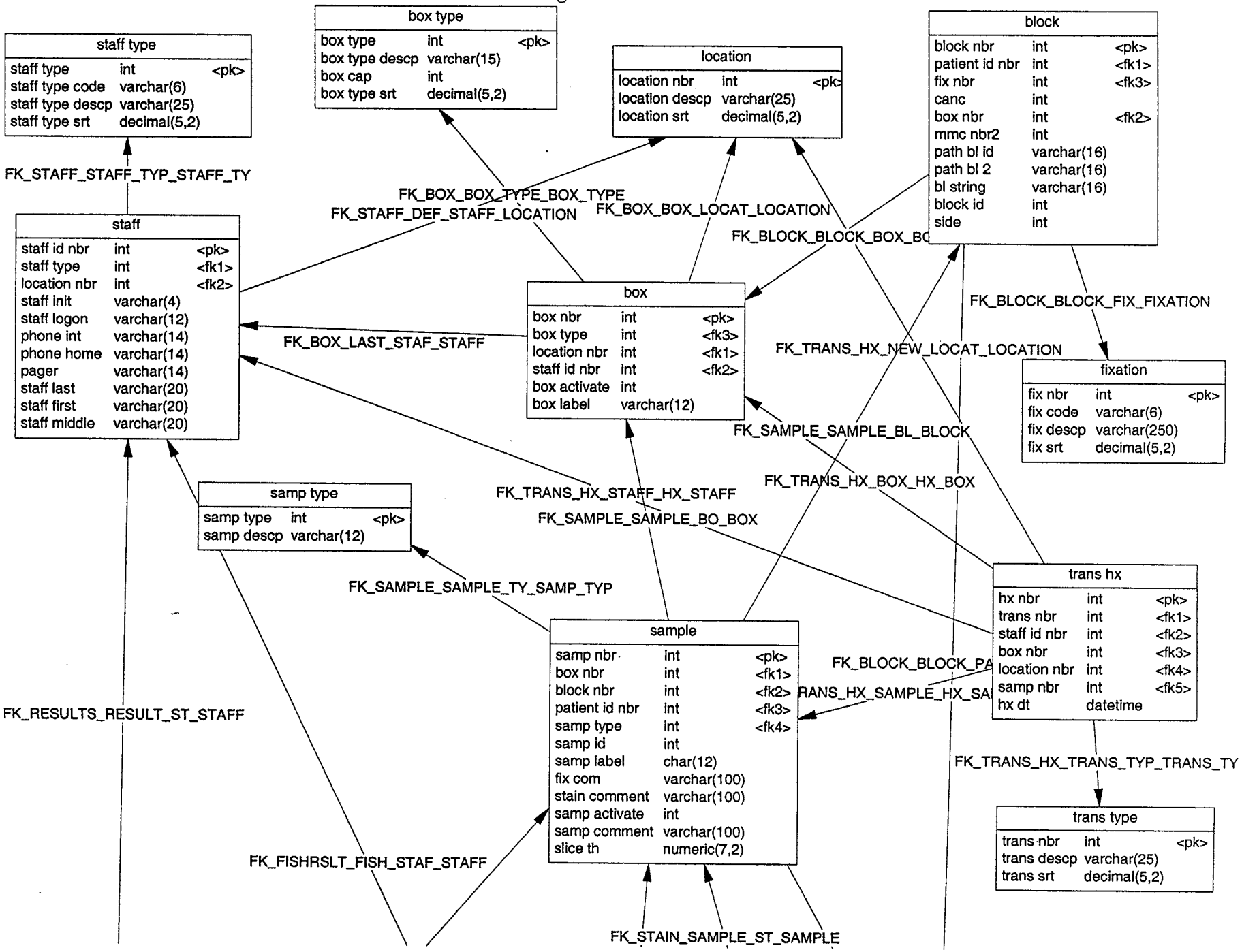
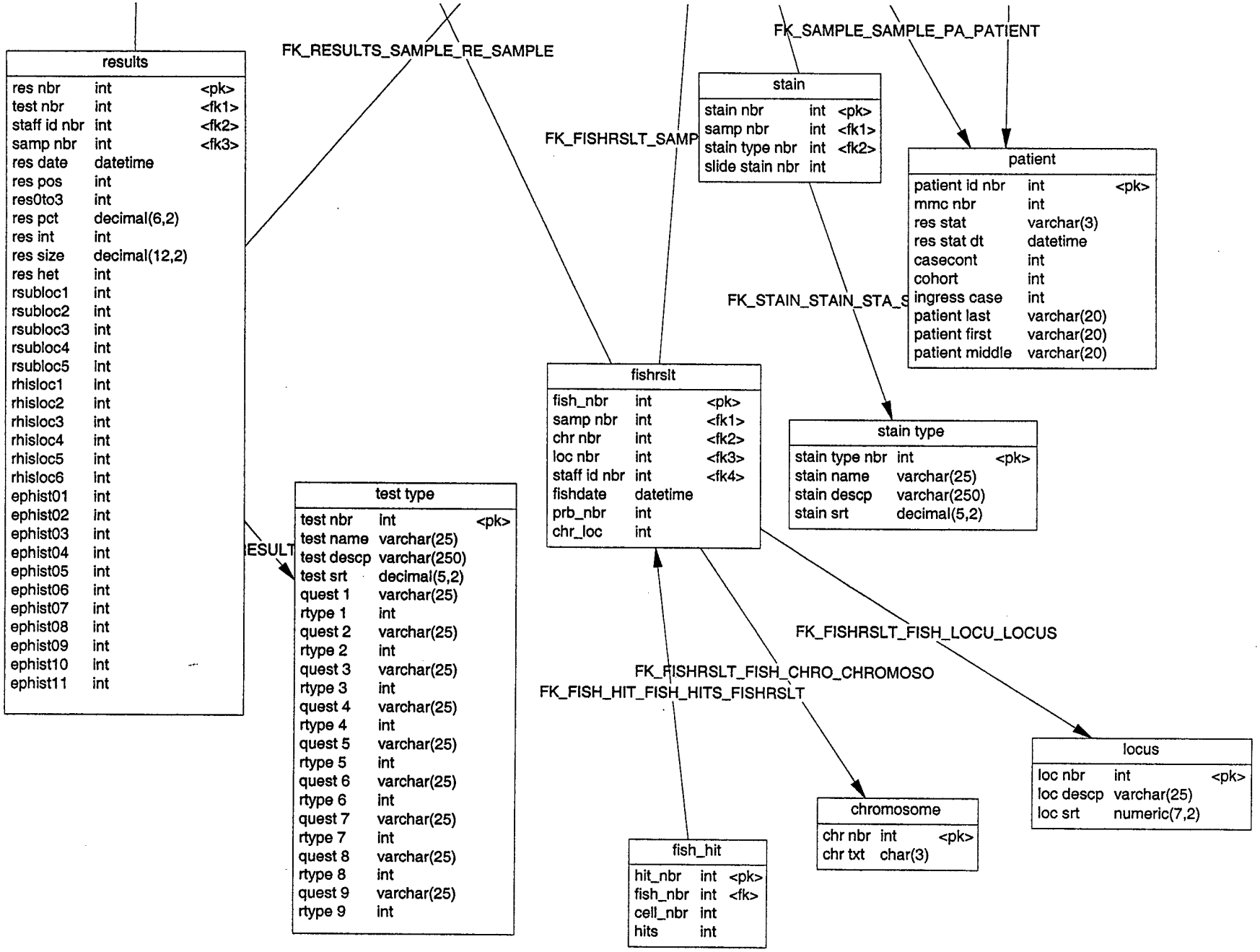


Figure 1. Examples of immunostains on representative specimens. 1A) COX–2 labeling of benign specimen from 1976. 1B) Cyclin D2 labeling of benign specimen from 1970. 1C) Dual labeling of Her2 (red) and p53 (brown) in cancer specimen from 1998. 1D)Fluorescence gamma tubulin (red) labeling of benign specimen from 1981. Nuclei are counterstained with hematoxylin (1A–C) and Hoechst 33342 (1D).

Figure 2

Components of Relational Database (6/03)
Figure 2

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